

Correspondence

Reply

To the Editor:

Amaral¹ has raised some important questions that we are pleased to address regarding our recent work on the urinary metabolic profile of patients with exacerbated asthma.²

Recently, evidence has shown that oxidative stress is increased in asthmatic patients, either through depletion of antioxidants or accumulation of reactive oxygen species (ROS), with effects on DNA structure, modification of proteins and lipids, and production of proinflammatory and anti-inflammatory cytokines.³ In a contemporary article by Zuo et al,⁴ the relationship between molecular mechanisms of asthma and pulmonary inflammation in asthmatic patients was reviewed, making clear that pulmonary ROS formation is a component of the molecular mechanism of asthma, resulting in physiologic damage. Nevertheless, it is reasonable to expect that pulmonary ROS formation can aggravate inflammatory diseases, such as asthma, and be a consequence of it, in a vicious circle.

The confounding effect of corticosteroid use has been addressed in previous studies directed at nuclear magnetic resonance metabolomics analysis in asthmatic patients in which it was eliminated.⁵ The obtained results pointed in the same direction as ours because the identified metabolites in the sera of asthmatic patients were related to hypermethylation, response to hypoxia, and immune reaction.⁵ In addition, the fact that our patients were treated with similar corticosteroid doses dilutes this confounding effect.

The use of nonfasting samples was intentional because our aim was to find urinary metabolic changes in exacerbations in real-life patients, in whom we cannot choose the time of the day to exacerbate. As stated in our study, the use of 2 techniques enhances our results: if some of the metabolites found using the ¹H nuclear magnetic resonance technique can be related to diet, others, such as citrate and alanine, suggest a disturbance of the tricarboxylic acid cycle or carnitine and acetylcarnitine that can explain the increase in oxidative burden because they play an essential role in the transport of fatty acids into mitochondria. Also, alkanes and aldehydes identified by using 2-dimensional gas chromatography, levels of which were found to be increased in exacerbations, are end products of the peroxidation of unsaturated fats. These metabolites might also be related to the oxidative processes of the arachidonic acid cycle. Isoprostanes, bioactive prostaglandins produced independently through the peroxidation of arachidonic acid in the presence of ROS, have also been implicated by other authors in asthmatic patients.⁶

We agree with Amaral¹ that sample size is a limitation. Given the small sample numbers, we have applied principal component

analysis to the data, a method in which class membership is not included in the modeling process, and we have purposely avoided supervised methods, such as partial least-squares discriminant analysis, which would carry a high risk of overfitting. We have also emphasized the advantages of detecting multivariate profiles as possible markers (rather than individual metabolite markers).

We are now able to add that we have data (not yet published) comprising a larger and different sample set that also point out that lipid peroxidation is involved in asthma to a different extent according to disease severity, making us believe that these preliminary published results were not due to hazard.

Cláudia Chaves Loureiro, MSc^{a,b}

Jean Bousquet, MD^c

Ana Todo Bom, PhD^b

Sílvia M. Rocha, PhD^d

From ^aServiço de Pneumologia, Hospitais da Universidade de Coimbra, Coimbra, Portugal; ^bthe Centre of Pneumology, Faculty of Medicine, University of Coimbra, Coimbra, Portugal; ^cUniversity Hospital Hôpital Arnaud de Villeneuve, Montpellier, France, and Inserm, CESP Centre for research in Epidemiology and Population Health, U1018, Respiratory and Environmental Epidemiology team, Villejuif, France; and ^dQOPNA, Departamento de Química, Universidade de Aveiro, Aveiro, Portugal. E-mail: cl_loureiro@hotmail.com.

Disclosure of potential conflict of interest: J. Bousquet has served on scientific and advisory boards for Actelion, Almirall, Meda, Merck, MSD, Novartis, Sanofi-Aventis, Takeda, Teva, and Uriach; serves on the Board of Directors for Stallergenes; and has received payment for lectures from Almirall, AstraZeneca, Chiesi, GlaxoSmithKline, Meda, Merck, MSD, Novartis, OM Pharma, Sanofi-Aventis, Schering Plough, Takeda, Teva, and Uriach. A. Todo Bom has consultant arrangements with Laboratórios Vitória, has received payment for lectures from GlaxoSmithKline and MSD, has received payment for development of educational presentations from Thermo Scientific, and has received travel support from Boehringer Ingelheim. The rest of the authors declare that they have no relevant conflicts of interest.

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